THE JEREMIAH METZGER LECTURE

INFLAMMATION, IMMUNE MODULATORS, AND CHRONIC DISEASE

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ABSTRACT

Chronic inflammation is a risk factor for many different diseases. It is clear that inflammation is associated with degenerative brain diseases, obesity, metabolic syndrome, cardiovascular disease, diabetes, and cancer. Throughout the past 100 years, changes in the causes of death in the US have been dramatic. The most recent data indicate that cardiovascular disease and cancer are now responsible for 63% of mortality in the US population. Although progression of these diseases is related to diet, lifestyle, and genetic factors, a common but often unrecognized link is the presence of underlying chronic inflammation. As of 2014, 83.6 million people were living with some form of cardiovascular disease, 29.1 million people have been diagnosed with diabetes, 14 million people carried the diagnosis of cancer, and 5.2 million people were living with Alzheimer disease. These diseases are a huge burden on our health care system and all have been associated with chronic inflammation.

INTRODUCTION

Inflammation is a protective tissue response to injury that destroys, dilutes, or walls off both the injurious agent and the injured tissue. The classic signals of acute inflammation include pain, heat, redness, swelling, and loss of function. Primarily, immune cells are responsible for inflammatory process and elaborate mediators, which cause the local reaction. The immune system is composed of both innate and adaptive responses. Innate immunity is present at birth and is not affected by an invader or pathogen; it has no memory. Its function is carried out by monocytes, macrophages, neutrophils, eosinophils, basophils, natural killer cells, dendritic cells, and the complement system.

Acquired or adaptive immunity is not present at birth and must be learned as a person's immune system encounters foreign substances

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(antigens). Acquired immunity is the basis for effective vaccines used to prevent both viral and bacterial infections while the body learns the best way to attack each antigen to prevent disease. Components of the acquired immune system are lymphocytes and antibodies.

Studies of tissue injury and inflammatory response date back to the early 1800s, when histopathology was first developed to understand the specific cells involved in this process. In the late 19th century, studies demonstrated that macrophages took up granulocytes at sites of inflammation to help clear the inflammatory cells. It was not until the 1940s that reports first appeared indicating that injured cells produce inflammatory mediators. In the 1960s, lymphocytes were first shown to kill cells. In 1975, tumor necrosis factor was first detected as a molecule that caused tissue injury. Over the past several decades, incredible advances have been made in understanding the molecular pathways and cells involved in the suppression and stimulation of an inflammatory response (1).

Cardiovascular Disease

On close examination, atherosclerotic plaques are composed of multiple immune cells and other components that indicate poor resolution of inflammation (2). These plaques often contain a necrotic core with secondary necrosis caused by defective efferocytosis. Efferocytosis is the process by which dying or dead cells are removed from the tissue environment by phagocytic cells (usually macrophages). Fat-laden macrophages seen in atherosclerotic plaques are known as *foam cells*. They are an indication of plaque buildup, which is commonly associated with an increased risk of heart attack and stroke. Foam cells with cholesterol deposits are present and unable to egress from the lesion and these cells release inflammatory cytokines. Monocytes are found to be present in these lesions as well. Essentially, atherosclerotic plaques contain an inflammatory microenvironment that causes tissue damage, and ultimately upon plaque rupture, they create a blockage in the vessel in which they reside. In progressing plaques, macrophages from the circulatory system first adhere to endothelial cells, then migrate across the endothelial surface and proliferate in the intima of the vessel before engulfing low-density lipoprotein and oxidized low-density lipoprotein to ultimately become foam cells with defective efferocytosis. In regressing plaques, these foam cells are able to unload their lipids and ultimately undergo reverse transmigration or effective efferocytosis, which helps to clear the lesion. In advanced atherosclerosis, the foam

cells undergo endoplasmic reticulum stress and apoptosis, but ultimately are not cleared from the intima. They then undergo secondary necrosis, which leads to the release of pro-inflammatory factors and necrotic core formation.

Metabolic Disease, Adipokines, and Inflammation

The worldwide epidemic of obesity has drawn considerable attention to research aimed at understanding the biology of adipocytes and the events occurring in fat tissue that contribute to disease processes (3). Adipose tissue releases multiple bioactive substances, referred to as *adipokines*, that have both pro-inflammatory and anti-inflammatory properties. Dysregulated secretion of these adipokines can contribute to the obesity-linked complications of a variety of diseases. Obesity is strongly associated with insulin resistance, hypertension, and dyslipidemia, which contribute to high rates of morbidity and mortality. Excess adipose mass is associated with increased levels of C-reactive protein (CRP) in the blood. Increased levels of CRP and interleukin-6 (IL-6) are predictive of the development of type 2 diabetes mellitus. Importantly, interventions that lead to weight loss lead to reductions in pro-inflammatory proteins including CRP and IL-6. The production of most adipokines is increased in the obese state, and these pro-inflammatory factors function to promote obesity-linked metabolic diseases.

Diabetes

Type I diabetes (T1D) is one of the most common diseases of childhood. It is thought to have an autoimmune/inflammatory component, and greater than 90% of individuals with T1D test positive for at least one autoantibody (4). The peak incidence of the disease is between 6 and 15 years of age, with a second peak occurring later in adolescence. Studies have shown that in response to viral infection, human islets secrete such pro-inflammatory cytokines as IL-6, IL-8, tumor necrosis factor, and chemokines such as CXC chemokine 10 (CXCL10), CC chemokine 3 (CCL3) and CCL4. Essentially, this immune reaction targets the β cells in the pancreas, thus eliminating their ability to produce insulin. Numerous studies have implicated cells of the innate immune system in both the initiation and development of diabetes. Blockade of macrophage entry into the pancreas or inhibition of macrophage function in mice prevents the onset of diabetes, suggesting a key role for these cells in β-cell destruction. Cytokines produced by cells of the innate immune system have been implicated in β-cell

dysfunction in the pancreas of mice and humans. T1D is a complex disease that is influenced by genetic and environmental factors and clearly involves the innate and adaptive arms of the immune system.

Alzheimer Disease

Ninety-six percent of individuals diagnosed with Alzheimer disease are older than 60 years of age. Some of the risk factors for Alzheimer disease include reduced physical activity or a sedentary lifestyle, midlife obesity, sepsis or systemic inflammation, and poor oral health or periodontitis. All of these situations lead to a systemic increase in pro-inflammatory mediators. This, along with certain genetic factors, can lead to reduced clearance of β-amyloid, which increases the number of activated microglial cells and neuron damage (5). The NLRP3 inflammasome is activated in Alzheimer disease and contributes to its pathology in certain mouse models. Inflammatory mediators that result from NLRP3 inflammasome activation are probably involved in mediating synaptic dysfunction, cognitive impairment, and the restriction of beneficial microglial clearance functions. This key role of the NLRP3 inflammasome in amyloid-β–mediated inflammatory responses suggests the possibility that a drug that blocks the activity of the NLRP3 inflammasome, or inflammasome-derived cytokines, might effectively interfere with the progression of Alzheimer disease. This is consistent with the hypothesis that amyloid-β–induced activation of the NLRP3 inflammasome enhances Alzheimer disease progression by mediating a harmful chronic inflammatory tissue response.

Cancer

Genomics and genetics have played a major role in unlocking the mysteries of cancer throughout the past two decades. However, less than 10% of all cancers are caused by germline mutations. The remainder are caused by acquired somatic mutations and environmental factors. In many cases, cancer initiation and progression can be linked to chronic infection, dietary factors, obesity, inhaled pollutants, tobacco use, or autoimmunity (6). The common theme among all these situations is chronic inflammation. Hence, malignancies may be viewed as a situation whereby inflammatory and neoplastic processes co-develop into a “wound that does not heal.” Rudolph Virchow first linked inflammation to cancer in the 1800s when he described the presence of leukocytic infiltrates in the tumor microenvironment. As much as 20% of cancer cases worldwide can be attributed to some sort of viral or bacterial infection. Recent studies have indicated that vari-

ations in the microbiota are associated with inflammation and cancer development. More work is underway to identify the specific components of the microbiome involved and to understand the specific mechanisms responsible. Inflammation is causally related to cancer in ways that involve genotoxicity, aberrant tissue repair, cell proliferation, invasion, and metastasis. Malignant cells can modulate the inflammatory environment by the secretion of soluble factors and chemoattractants that make inflammatory cells suppress the anticancer T-cell responses. In some cases, the chronic inflammatory response is caused by the presence of an infectious agent, and in others the inflammation is stimulated by the presence of a developing cancer, which in concert suppresses the ability of cytotoxic T cells to kill the carcinoma cells.

DISCUSSION & SUMMARY

Inflammation is our first defense against infection, but when it goes awry in specific organ sites, it can lead to progression of several diseases, including cancer, cardiovascular disease, metabolic syndrome, diabetes, and Alzheimer disease. When an individual undergoes acute tissue damage through blunt trauma or other means, the resulting swelling and inflammation is obvious, painful, and short-lived. The immune system reacts immediately by sending in lymphocytes and other inflammatory mediators to help remove the damaged tissue and start the healing process. Inflammation is a critical component of the immune system to help fight off viruses, bacteria, fungi, and other invaders. Inflammation can become chronic when there is a persistent stimulus without resolution.

This Metzger lecture was focused on delineating the connections between chronic inflammation and a variety of diseases. Oftentimes, medical treatments are focused on deficiencies related to end-organ damage, with little concern or understanding about the pathophysiologic changes that underlie tissue damage or organ dysfunction. This lecture provided some clear connections among inflammation and cardiovascular disease, metabolic syndrome, obesity, diabetes, and cancer—diseases that are currently responsible for the majority of deaths in the US. Future efforts need to be directed at understanding effective ways to curb chronic inflammatory pathways to prevent morbidity and premature death.

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REFERENCES

1. Jones DS, Podolsky SH, Greene JA. The burden of disease and the changing task of medicine. *N Engl J Med* 2012;366:2333–28.
2. Moore KJ, Sheedy FJ, Fisher ER. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol* 2013;13:709–21.
3. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011;11:85–97.
4. Herold KC, Vignali DAA, Cooke A, Bluestone JA. Type 1 diabetes: translating mechanistic observations into effective clinical outcomes. *Nat Rev Immunol* 2013;13:243.
5. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nat Rev Immunol* 2014;14:463–77.
6. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat Rev Cancer* 2013;13:759–71.

DISCUSSION

Crawford, Nashville: One of the questions that we always ask ourselves in thinking about new treatments, is it better than the old ones? Is it better than aspirin? If you treat the animals with aspirin, do you have the same kind of counter-regulatory effects that you would expect from the PGE2 experiments on the CXCR2 and CXCL ligands?

DuBois, Tempe: Obviously we have to keep that in our mind in terms of the expense and other side effects of these newer treatments that may be developed. In the animals, treating mice with aspirin is a problem, because they deal with aspirin much differently than humans do. But it does have some activity. But if you treat those mice with celecoxib, for example, which is handled much better pharmacokinetically in the mice, it is very effective at doing the same thing as the CXCR2. I think the CXCR2 pathway is also affected by other non-prostaglandin–dependent modulators, and it may be something that could be explored in some situations and individuals with advanced disease. I don't think it is a way to prevent disease. But certainly there may be up to 20% of colorectal cancers that are stage 4 that there might be a role there. There are some early-phase studies underway. So we'll see if that holds up.

Williams, Boston: Given all the work that has been done on aspirin and cancer and heart disease, has anyone looked at the effect of aspirin on Alzheimer's?

DuBois, Tempe: There has been some epidemiologic work, and actually there were some positive studies that were reported with NSAIDs and aspirin on Alzheimer's. It's not as dramatic as in colorectal cancer. There are some groups that are working on that to see if it is something that could be developed. Certainly something that could modulate that neuroinflammatory response, I think, could be beneficial.

Williams, Boston: Since microglia are thought to be derived as a myeloid derivative of hematopoietic stem cells, have you looked in your model yet at models of brain inflammation?

DuBois, Tempe: We haven't, but I think that is actually a very good idea. We don't even know how to get to the brain. We really have been working on the intestines. So it is something that we are going to have to collaborate with somebody on.

Tweardy, Houston: I was curious to know if you had some thought about CXCL receptor antagonists that are being developed. What would be your first entry for instance into the clinic with those types of agents?

DuBois, Tempe: Well I am biased towards GI cancers. I know it is being developed for some other things as well. There are also some monoclonal antibodies that are being developed for that receptor. I would like to see in some of these preclinical models what kind of activity there is. I think you could go fairly quickly into stage 4 colorectal cancer. There is [a] study now underway in Seattle at the University of Washington looking at capecitabine plus celecoxib in one of the arms of that trial, and they are getting some significant clinical responses in about 20 to 30% of the patients. That might be a good place to look at that initially in early phase 1 and phase 2 studies.

Schuster, New York: In the experiments you showed with topical application of PGE2 to the colon, were those wild-type mice or azoxymethane primed or 15 dehydrogenase nulls? What was the background there?

DuBois, Tempe: That is a very good question. What you have to do to develop this model is to have the adenomatous polyposis coli suppressor gene deleted or mutated in one allele. One of the alleles of that suppressor gene is affected. They are the APC Min mice. Then we give azoxymethane to those mice as well, and then give the dextran sulfate 7 days on, 7 days off for that protocol. I am happy to send that to you if you are interested.

Alexander, Atlanta: The notion of multiple causes or multiple phenotypes arising from similar molecular mechanisms is something that is very much talked about recently. A fair amount of that has looked at it from the point of aging and senescence. P53 is very prominent pathway, and other pathways of senescence and organismal aging are beginning to be sorted out. Are we looking at taking different views of the same thing?

DuBois, Tempe: I think that is a very good point. People get sort of pigeon-holed into their focus on an individual disease. I think that one of the eye-opening things for me by moving to Arizona and taking on the role as leading the Institute is that when you bring in several different points of view to a particular problem, you do get a slightly different view of it than just looking at it the same old way. It's clear that in these different diseases and the different tissue microenvironments, it's not all the same molecular pathways or immune cells involved. What I am thinking—and hopefully will see moving forward—is that by learning specifically what those interactions are and what the inciting agents are, there may be some commonality among the immune system and some immune modulation, not so much immune suppression or whatever. But there are more sophisticated ways now being developed that we can modulate some of these subtypes of the immune system that could be utilized and play a role. Clearly one of the things that is in common with all of these diseases that I discussed is that if you have a healthy lifestyle with exercise, a good diet, all these things . . . it reduces the amount of that inflammation and other factors in the tissue microenvironments. So there are some common denominators from that wellness perspective. Now the question is, will any medicines or drugs be developed that can be used to modulate that in individual patients? Clearly that is an open question I think.